



Prospects of indigenous fungi as novel biological control agents of thrips (*Frankliniella occidentalis* Pergande, 1895) on tomato (*Solanum lycopersicum* L.) in vivo

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Abstract

Tomato (*Solanum lycopersicum* L.) is an important horticultural crop in Kenya. Its production is constrained by many factors, among them arthropod pests and diseases. In response, farmers rely on synthetic pesticides, which lead to contamination of the produce, pest resistance and pollution of the environment hence there is need to identify safer, affordable alternatives. Biological control is considered safe, self-sustaining and cost effective. This study was aimed at determining the effectiveness of indigenous fungi in managing Western flower thrips *Frankliniella occidentalis* on tomato. Efficacy trials were conducted in farmer's fields in Bungoma County, Kenya between March and November 2018. Treatments included fungal isolates *Trichoderma harzianum*, *Gliocladium virens*, *Verticillium* spp., *Paecilomyces victoriae* and *Fusarium oxysporum* selected after *in vitro* screening. These were compared to commercial fungus *Beauveria bassiana*, a synthetic pesticide imidacloprid and untreated control. Treatments were replicated four times, arranged in a randomized complete block design. Data collected on population of thrips and the yield of tomatoes were subjected to Analysis of Variance (ANOVA). Means were separated using Student Newman-Keuls (SNK) test at $p \leq 0.05$. In the first season at Bukonoi, *F. oxysporum* significantly ($p < 0.05$) recorded the least (38.1) mean number of thrips compared to the untreated control (89.2). At Cheptais, *F. oxysporum* and *T. harzianum* treated plots significantly ($p < 0.05$) recorded the least mean number of thrips of 48.1 and 20.5 in the first and second season, respectively. Higher yields of 4.9 t ha⁻¹ and 29.5 t ha⁻¹ were obtained from plots treated with *T. harzianum* in the first and second season, respectively. The findings of this study demonstrated that *F. oxysporum* and *T. harzianum* have the potential to be developed as fungal biopesticides for management of thrips on tomato crop. However, large scale field trials are warranted to validate the effectiveness of these fungal isolates.

Key words: Biopesticides, *Frankliniella occidentalis*, *Fusarium oxysporum*, *Solanum lycopersicum*, *Trichoderma harzianum*.



Introduction

The Western flower thrip, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important quarantine arthropod pest of horticultural crops in the world (Infonet-Biovision, 2022). Thrips cause abscission of buds, flowers and deformation of fruits. Thrips are also potential vectors of viral diseases such as Tomato Spotted Wilt Virus and the Tomato Chlorotic Spot Virus (Infonet-biovision, 2022). Despite their negative impacts on living organisms and the environment, synthetic pesticides have been extensively used to manage thrips (Ndakidemi *et al.*, 2016). Frequent application of such pesticides has led to development of resistance (Wagnitz, 2014). The numerous negative effects associated with chemical pesticides have led to increased interest in developing environmentally safer and sustainable strategies to manage arthropod pests. In particular, focus has been on the use of biopesticides as an alternative to synthetic pesticides in integrated pest management strategies (Srijita, 2015).

Biopesticides have several advantages including less toxic residues, safety to non-target organisms, varied modes of action on pests, host specificity, compatibility with other pest management strategies, sustainability and affordability to farmers if produced locally (Ouma *et al.*, 2014). This study investigated the effectiveness of local antagonistic fungi against *F. occidentalis* on tomato under field conditions as alternatives to synthetic pesticides for reduced chemical residues in the produce.

Materials and methods

Description of study sites

Field trials were carried out for two tomato growing seasons in farmers' fields at Bukonoi and Cheptais in Bungoma County, Kenya. The first season was carried out between March and July 2018 while the second season was carried out between August and November 2018. Bukonoi is located at 0°48'36''N and 34°28'12''E, 1,635m above sea level (masl) with volcanic soils while Cheptais



is located at 0°48'0''N, 34°27'36''E, 1,593 masl with sandy clay loam soils. The County receives bimodal rainfall ranging from 950 to 1500 mm per annum with the long rains being experienced between March to July and short rains between August to November (Jaetzold *et al.*, 2012). The average annual temperatures range from 15 to 23°C (NAFIS, 2018; Bungoma CIDP, 2013-2018).

Source of fungal isolates

The fungal antagonists evaluated were *Gliocladium virens*, *Verticillium* spp., *Trichoderma harzianum*, *Paecilomyces victoriae* and *Fusarium oxysporum*. The antagonists were originally isolated from soil samples collected from Bungoma County, Kenya in rhizosphere of tomato plants. These were selected based on their virulence against *Frankliniella occidentalis* *in vitro* experiments (Barasa *et al.*, 2021). They were mass produced on sorghum (*Sorghum bicolor* L.) grains since it is locally available and has shown good performance (Kumar *et al.*, 2014). Two hundred grams of sorghum were

weighed and washed properly three times using fresh tap water. The sorghum grains were pre-cooked by soaking in boiled water for 20 minutes. The substrate (sorghum) was placed into Milner bags (30 x 65cm) and autoclaved for 15 minutes at 121°C then cooled to about 40-45°C. The aerated sorghum bags were inoculated with 3-day old fungal broth and incubated at room temperature. During the incubation period, contents of the polythene bags were manually shaken vigorously every two days to prevent clumping and improve aeration as described by Sivakalai and Ramanathan (2015). After 14 days, the substrate was transferred into sterile plastic trays (25cm x 20cm) covered with a serviette on top and tied with a rubber band. They were allowed to air dry for 10 days at room temperature and conidia were harvested using a sieve (295µm pore mesh size) (Gathage *et al.*, 2016).

One-gram sample of each fungal antagonist was weighed and suspended in 9ml distilled water in separate



universal bottles. The suspension was shaken in a mechanical shaker (Unimax 1010) for 5 minutes and filtered through double layered muslin cloth. The concentration of fungal antagonists was standardized through serial dilution to 1×10^8 conidia g^{-1} . Harvested conidia were packed in sealed polythene bags (30cm x 20cm), labeled and stored at 4°C ready for use in the field.

Crop establishment and trial design

Seeds of Rio-Grande tomato variety were established in a nursery bed. The experimental field (39m by 15m) was manually prepared using a hoe. The field was divided into 32 plots, each measuring 4m by 3m with 1m buffer zones between the plots. The experiment was laid out in a randomized complete block design (RCBD) with eight treatments replicated four times. Four-week-old healthy tomato seedlings from the nursery were transplanted at a spacing of 60cm x 30 cm (Infonet-biovision, 2022), making a total of 67 plants per plot. Planting holes were dug per plot and 10g Diammonium phosphate

fertilizer was thoroughly mixed with the soil. The seedlings were placed and watered immediately to enhance establishment. Gapping of dead seedlings was done one week after transplanting. Calcium Ammonium Nitrate (CAN) was applied as top dressing at a rate of 10g per plant at fourth week after transplanting. Weeding was done at 3, 6 and 9 weeks after transplanting.

Application of treatments

Treatments application commenced at the sixth week after transplanting of tomatoes. The tomato plants were at flowering stage (BBCH 61). Subsequent treatments were applied every 7 days as foliar sprays. The test fungal isolates *Gliocladium virens*, *Verticillium* spp., *Trichoderma harzianum*, *Paecilomyces victoriae*, *Fusarium oxysporum* were applied at concentration of 1×10^8 conidia g^{-1} . These were compared with registered commercial fungus Bio-power (*Beauveria bassiana*) at recommended dose rate of 100g/20 litres of water, synthetic insecticide Confidor 70 WG®



(Imidacloprid 700g/Kg) at 5g/20 litres of water and a negative control (water).

Each concentration (1×10^8 conidia g^{-1}) of *Gliocladium virens*, *Verticillium* spp., *Trichoderma harzianum*, *Paecilomyces victoriae*, *Fusarium oxysporum* was mixed with water and then sprayed using a calibrated knapsack sprayer CP 15s (Cooper Pegler and Co. Ltd, Sussex, England) with a flat cone nozzle. Application was done starting with the negative control (water) followed by the test fungal isolates, the standard bio-pesticide and the standard synthetic pesticide imidacloprid, respectively. Spraying was done in the evening between 16:00h and 18:30h to lessen the adverse effects of ultraviolet radiation (Mustafa & Kaur, 2010). During treatment applications, polythene sheets were used to avoid drift of mist to neighboring plots. The knapsack sprayer was thoroughly washed with water and soap, rinsed three times before use in spraying each of the treatments. The treatments were applied in their

respective plots five times during the trial period.

Data collection

Assessment of pest population

The assessment of thrips population was conducted at early hours of the day between 7.00-10.00am as described by El-Shafie and Abdelraheem (2012). This was done five times at flowering from 6th to 10th week after transplanting of tomatoes. Twenty well developed flowers from 20 randomly tagged tomatoes in the inner rows per plot were cut and placed in high density plastic poly pots (35ml) containing 70% ethanol. Each flower was placed in a petri dish, dissected and rinsed with water making sure that no thrip was washed off. The number of thrips were counted using a tally counter under the dissecting microscope (NTB-3A) at $\times 10$ magnification and recorded.

Assessment of tomato yield

At maturity (12th week after transplanting) tomato fruits at pink stage were harvested in whole plots. The produce was graded into marketable and



non-marketable categories. The weight of tomatoes in kilograms was determined using a digital hand-held electronic scale and extrapolated into tons ha^{-1} as follows: $Y = (W \times 10,000) \div A$, where, Y is the yield in tons ha^{-1} ; W is the total weight in tons of harvested tomatoes and A is the plot size in m^2 (Ashenafi *et al.*, 2017).

Statistical analysis

Data on number of thrips was checked for normality using Shapiro-Wilk test and were normalized using square root transformation $[\text{SQRT}(x+1)]$ before analysis. The transformed data on thrips and yield of tomatoes were subjected to one-way ANOVA using SAS version 9.1 (SAS Institute, 2013). Post-hoc test was conducted using the Student–Newman–Keuls test (SNK) and means were separated at $p < 0.05$ (Sokal & Rohlf, 1995).

Results

In Bukonoi, thrip population in plots treated with fungal isolates were significantly lower ($p < 0.05$) compared to populations observed in control plots.

over the two trial seasons. In the first season (March–July 2018), plots treated with imidacloprid recorded the least population density of thrips (27.2) and its effect was not significantly different from that recorded in the fungal isolates *G. virens*, *T. harzianum*, *P. victoriae* and *F. oxysporum*. Of the evaluated isolates, plots treated with *F. oxysporum* recorded the least population density of thrips (38.1) and its effect was not significantly different from plots treated with the rest of the fungal isolates and the commercial fungal biopesticide (*B. bassiana*). Control plots recorded the highest population density of thrips (89.2) which was significantly ($p < 0.05$) different from the rest of the treatments (Table 1).

In the second cropping season (August–November 2018), a similar trend was observed. Plots treated with imidacloprid recorded the least population density of thrips (17.4) and its effect was not significantly different from plots treated with *Verticillium* spp., *T. harzianum*, *F. oxysporum* and the standard commercial fungus (*B. bassiana*). Of the evaluated isolates, *Verticillium* spp. treated plots



recorded the least population of thrips (17.9) followed by *F. oxysporum* and *T. harzianum* with 18.2 and 18.3 thrips, respectively. Control plots recorded the

highest population density of thrips (32.3) which was significantly ($p < 0.05$) different from the rest of the treatments (Table 1).

Table 1: Number of thrips on tomatoes sprayed with different treatments during the two cropping seasons at Bukonoi.

Mean no. thrips/20 flowers \pm S.E			
Treatment	Rate/concentration	Season 1 (March-July 2018)	Season 2 (August-November 2018)
<i>G. virens</i>	1x10 ⁸ conidia g ⁻¹	43.8bc	22.3b
<i>Verticillium spp.</i>	1x10 ⁸ conidia g ⁻¹	46.7b	17.9cd
<i>T. harzianum</i>	1x10 ⁸ conidia g ⁻¹	44.1bc	18.3cd
<i>P. victoriae</i>	1x10 ⁸ conidia g ⁻¹	44.4bc	21.2bc
<i>F. oxysporum</i>	1x10 ⁸ conidia g ⁻¹	38.1bc	18.2cd
<i>B. bassiana</i>	100g/20l of water	54.7b	16.7d
Imidacloprid	5g/20l of water		
700g/Kg		27.2c	17.4d
Control (water)	-	89.2a	32.3a
<i>p</i> -value		0.0015	<0.0001
<i>F</i> -value		8.6	18.6

Means followed by the same letter (s) in each column are not significantly different according to Student Newman-Keuls (SNK) test at $p < 0.05$.

At Cheptais, the evaluated fungal isolates significantly ($p < 0.05$) reduced the population of thrips compared to the control for the two trial seasons. In the first cropping season (March-July 2018), plots treated with the standard insecticide (imidacloprid) recorded the

lowest population density of thrips (28.5) followed by *F. oxysporum* and *T. harzianum* treated plots with 48.1 and 48.8 thrips, respectively. The effect of the evaluated fungal isolates *Verticillium spp.*, *T. harzianum*, *P. victoriae* and *F. oxysporum* in reducing thrips population



was not significantly different from *B. bassiana*. The control plots recorded the highest population density of thrips (90.5) which was significantly different ($p < 0.05$) from the rest of the treatments (Table 2).

During the second cropping season (August-November 2018), plots treated *T. harzianum* recorded the least

population density of thrips (19.1) and its effect was not significantly different from the fungal isolates *Verticillium Spp.*, *P. victoriae*, *F. oxysporum* and imidacloprid. The control treated plots recorded the highest population density of thrips (35.3) which was significantly ($P < 0.05$) different from the rest of the treatments (Table 2).



Table 2: Number of thrips on tomatoes sprayed with different treatments during the two cropping seasons at Cheptais

Treatment	Rate/concentration	Mean no. thrips/ 20 flowers±S.E	
		Season 1 (March-July 2018)	Season 2 (August-November 2018)
<i>G. virens</i>	1x10 ⁸ conidia g ⁻¹	65.9b	27.1b
<i>Verticillium spp.</i>	1x10 ⁸ conidia g ⁻¹	50.9c	20.0bc
<i>T. harzianum</i>	1x10 ⁸ conidia g ⁻¹	48.8c	19.1c
<i>P. victoriae</i>	1x10 ⁸ conidia g ⁻¹	51.9c	21.5bc
<i>F. oxysporum</i>	1x10 ⁸ conidia g ⁻¹	48.1c	20.5bc
<i>B. bassiana</i>	100g/20l of water	55.4c	27.3b
Imidacloprid 700g/Kg	5g/20l of water	28.5d	26.1bc
Control (water)	-	90.5a	35.3a
<i>p</i> -value		0.0088	0.0007
<i>F</i> -value		5.3	10.0

Means followed by the same letter (s) in each column are not significantly different according to Student Newman-Keuls (SNK) test at $p \leq 0.05$.

During the first season at Bukonoi, the evaluated fungal isolates significantly ($p < 0.05$) improved the yield of tomatoes compared to the control. The standard synthetic insecticide imidacloprid 700g/Kg -treated plots achieved the highest total (4.9t/ha) and marketable (3.7t/ha) yield of tomatoes followed by plots treated with *T. harzianum* and *B. bassiana*. The lowest yield was recorded from the negative control plots (Table 3). In the second season, except for *T.*

harzianum-treated plots, the evaluated fungal isolates showed no significant effect on the yield of tomatoes compared to the control plots. Plots treated with *T. harzianum* achieved the highest total (29.5t/ha) and marketable yield (27.4 t/ha) of tomatoes which was significantly ($p < 0.05$) different from that achieved in the control plots. The control treated plots recorded the lowest yield (Table 3).



Table 3: Mean yield (t/ha) \pm SE of tomatoes harvested for the two seasons at Bukonoi

Treatment	Rate/concentration	Season 1 (March-July 2018)		Season 2 (Aug-Nov 2018)	
		Total	Marketable	Total	Marketable
<i>G. virens</i>	1x10 ⁸ conidia g ⁻¹	2.3 \pm 0.2bc	1.9 \pm 0.2b	24.1 \pm 3.1ab	21.8 \pm 2.8ab
<i>Verticillium spp.</i>	1x10 ⁸ conidia g ⁻¹	3.7 \pm 1.1b	3.0 \pm 1.0ab	26.8 \pm 4.3ab	23.9 \pm 4.1ab
<i>T. harzianum</i>	1x10 ⁸ conidia g ⁻¹	4.9 \pm 0.8ab	3.7 \pm 0.8ab	29.5 \pm 2.3a	27.4 \pm 2.3a
<i>P. victoriae</i>	1x10 ⁸ conidia g ⁻¹	3.5 \pm 0.3bc	2.8 \pm 0.3ab	25.7 \pm 2.6ab	23.2 \pm 2.5ab
<i>F. oxysporum</i>	1x10 ⁸ conidia g ⁻¹	2.1 \pm 0.3bc	1.6 \pm 0.2b	26.9 \pm 2.9ab	24.5 \pm 2.7ab
<i>B. bassiana</i>	100g/20l of water	4.5 \pm 1.8ab	3.4 \pm 1.6ab	27.0 \pm 2.6ab	24.0 \pm 3.8ab
Imidacloprid 700g/Kg	5g/20l of water	6.8 \pm 0.4a	4.9 \pm 0.4a	28.3 \pm 4.3a	25.0 \pm 4.2ab
Control (water)	-	0.0 \pm 0.0c	0.0 \pm 0.0c	18.2 \pm 1.8b	16.4 \pm 1.8b
<i>p</i> -value		0.0020	0.0150	0.0362	0.0429
<i>F</i> -value		5.1	3.2	1.1	1.0

Means followed by the same letter (s) in each column are not significantly different according to Student Newman-Keuls (SNK) test at $p \leq 0.05$.



During the first season at Cheptais, the evaluated fungal isolates significantly ($p < 0.05$) improved the yield of tomatoes compared to the control. The plots treated with imidacloprid 700g/Kg achieved the highest total (1.9 t/ha) and marketable (1.2 t/ha) yield of tomatoes followed by plots treated with *G. virens* and *P. victoriae* with marketable yield of 0.6 t/ha and 0.5 t/ha, respectively. The lowest yield was recorded from the negative control plots (Table 4). In the

second season, the evaluated treatments showed no significant effect on the yield of tomatoes compared to control plots. However, plots treated with *Verticillium spp.* recorded the higher total (18.3t/ha) and marketable (14.9t/ha) yields of tomatoes followed by the *T. harzianum* and *F. oxysporum*-treated plots. The lowest yield total (9.5t/ha) and marketable yield (7.0t/ha) was recorded from control plots (Table 4).



Table 4: Mean yield (t/ha) \pm S.E of tomatoes harvested for the two seasons at Cheptais.

Means followed by the same letter (s) in each column are not significantly different according to Student Newman-Keuls (SNK) test at $p \leq 0.05$.

Treatment	Rate/concentration	Season 1 (March-July 2018)		Season 2 (Aug-Nov 2018)	
		Total	Marketable	Total	Marketable
<i>G. virens</i>	1x10 ⁸ conidia g ⁻¹	0.9 \pm 0.1b	0.6 \pm 0.0b	13.8 \pm 5.1a	11.7 \pm 4.4a
<i>Verticillium spp.</i>	1x10 ⁸ conidia g ⁻¹	0.8 \pm 0.2b	0.4 \pm 0.2b	18.3 \pm 3.9a	14.9 \pm 3.4a
<i>T. harzianum</i>	1x10 ⁸ conidia g ⁻¹	0.7 \pm 0.3b	0.4 \pm 0.2b	17.9 \pm 1.4a	12.9 \pm 1.9a
<i>P. victoriae</i>	1x10 ⁸ conidia g ⁻¹	0.8 \pm 0.3b	0.5 \pm 0.2b	14.7 \pm 2.4a	11.4 \pm 1.9a
<i>F. oxysporum</i>	1x10 ⁸ conidia g ⁻¹	0.7 \pm 0.3b	0.5 \pm 0.2b	15.9 \pm 4.0a	12.7 \pm 3.2a
<i>B. bassiana</i>	100g/20 L water	1.0 \pm 0.1b	0.6 \pm 0.2b	13.1 \pm 2.7a	10.6 \pm 2.0a
Imidacloprid 700g/Kg	5g/20L water	1.9 \pm 0.4a	1.2 \pm 0.4a	15.7 \pm 2.9a	12.0 \pm 2.2a
Control (water)	-	0.0 \pm 0.0c	0.0 \pm 0.0c	9.5 \pm 1.3b	7.0 \pm 0.7b
<i>p</i> -value		<0.0001	0.0006	0.0659	0.0685
<i>F</i> -value		7.4	5.5	0.7	0.6



Discussion

The evaluated fungal isolates reduced the population of thrips with *F. oxysporum* and *T. harzianum* being most effective. The efficacy of *F. oxysporum* and *T. harzianum* was comparable to that of the commercial fungus (*B. bassiana*) and the synthetic insecticide (Imidacloprid 700g/Kg) demonstrating that these isolates have similar ability as that of the commercial products in suppressing thrips population in tomatoes. These findings compare with those of other researchers who reported insecticidal effects of *F. oxysporum* and *T. harzianum* in reducing infestation of arthropod pests infesting tomato crop (Lakhdari *et al.*, 2016; Lengai, 2016; Caccavo *et al.*, 2022). *Fusarium oxysporum* and *T. harzianum* are thus good candidates for biopesticides that can be used in *F. occidentalis* management.

The low tomato yields recorded during first season compared to the second season could be attributed to higher thrips infestation of recorded during the first season. High infestation of tomato

plants by thrips has been known to lead to flower abortion resulting in low fruit development and consequently low number of fruits harvested (Infonet-biovision, 2022). These findings corroborate previous studies that have reported reduced populations of arthropod pests by application of fungal antagonists with remarkable increase in tomato yield (El-Shafie & Abdelraheem, 2012; Lengai, 2016).

Among the tested fungal antagonists, *T. harzianum* was more efficacious in reducing the population of thrips with a resultant increase in the yield of tomatoes in both study sites for the two cropping seasons. These findings agree with Caccavo *et al.* (2022) who reported significantly higher fruit yield of tomatoes following application of *T. harzianum* in management of arthropod pests in the field. The effect of *T. harzianum* on the yield of tomatoes can also be attributed to its ability to promote growth of plants as well as inducing resistance to pathogens (Mwangi *et al.*, 2009; Sawant, 2014).



Conclusions and recommendations

The findings of this study demonstrated that *F. oxysporum* and *T. harzianum* were most effective in reducing the population of *F. occidentalis* on tomato and also increased marketable yield of tomatoes. Therefore, *F. oxysporum* and *T. harzianum* are good candidates for development as fungal-based biopesticides for *F. occidentalis* management on tomato. More studies should be conducted to determine bioactive compounds of these antagonistic fungi. This will be useful in the formulation and consequent commercialization as biopesticides. The synergistic effects between these antagonistic fungi should also be investigated.

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